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13. ABSTRACT ***(Maximum 200 Words)***
This four-year study is a cost-efficient and timely longitudinal study of bone turnover markers in an ethnically diverse sample of mid-aged women as they experience the menopause transition. Building on the multi-site Study of Women's Health Across the Nation (SWAN), funded by the National Institutes of Aging and Nursing Research at the National Institutes of Health, this study analyzed already collected and stored specimens of serum to measure bone formation (using an immunoradiometric assay of osteocalcin) and stored urine specimens to measure bone resorption (using urinary N-telopeptide of type I collagen). These two measures will be combined with data from SWAN on bone density (spine, hip and femoral neck), ovarian aging (endogenous sex hormones and menstrual bleeding), medications, medical history, social and psychological assessments, and life style factors (exercise, diet, smoking, body mass) to address five research aims. To date, all bone marker assays have been completed, analyses to address the research aims are presented in the attached report. Work is underway on the corresponding manuscripts.

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Sonja M. McKinney 4/27/01

PI - Signature Date

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5. Introduction

A. Background

In the last few years, there have been a number of studies examining the potential utility of biochemical markers of bone turnover in humans. The following is a review of major papers published in the last 5 years, since the submission of our original contract application to the Department of Defense (DOD).

1. Use of bone turnover markers to predict changes in bone density in women not receiving anti-resorptive therapy.

A major aim of our contract with the Department of Defense was to determine if baseline measurements of bone formation, serum osteocalcin (OC), and bone resorption, urinary excretion of N-telopeptide (NTx), markers could predict subsequent changes in bone mineral density (BMD) in pre- and early perimenopausal women of multiple ethnicity who are participating the The Study of Women's Health Across the Nation (SWAN).

The ability of bone turnover markers to predict changes in bone density in women not receiving anti-resorptive therapy has been examined in a number of recent studies. In 226 early postmenopausal women (mean age 51 years) who were randomly assigned to receive either calcium supplementation alone (n=118) or calcium plus hormone replacement therapy (HRT) (n=108), bone loss was greatest in women with the highest urinary NTx and serum OC levels (1,2). Urinary deoxypyridinoline (DPD) and serum bone-specific alkaline phosphatase (BSAP) levels did not predict changes in BMD in calcium-treated women (1,2). Similarly, in the OFELY cohort, a population based study of 305 postmenopausal women aged 50-88, baseline levels of a variety of bone formation markers [serum OC and type I collagen N-terminal propeptide, (PINP)] and bone resorption markers (urinary N-telopeptide (NTx), urinary C-telopeptide (CTx), and serum CTx) were associated with rates of forearm bone loss (3,4). In a group of 295 women, followed for an average of 3.8 years, all of the measured resorption markers (urinary NTx, DPD, pyridinoline, and CTx) predicted bone loss from the total hip (but not from the femoral neck) (5). Serum OC, but not bone-specific alkaline phosphatase (BSAP), also predicted bone loss from the total hip. The sensitivity, specificity, and predictive values of each marker were quite low, however, so that the authors concluded that the tests have limited value in individual subjects (5) In contrast to the study by Rosen et al. (1), McClung et al. (6) found no relation between baseline levels of serum OC or urinary NTx and rates of bone loss from the hip or spine in 458 early postmenopausal women (age 45-59 years) (6). Similarly, Keen et al found no relation between spine or hip BMD (measured by dual-photon absorptiometry) and multiple measures of bone turnover (using older assay methods) in 141 early postmenopausal women over a 4-year period (7). However, while Chung et. al. found no association with levels of serum OC or urinary DPD with femur BMD, they did find an association of serum OC and lumbar BMD in women 0 to 10 years after menopause (8). Finally, in women in the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial who received placebo, baseline individual bone turnover markers explained less than 5% of the variability in the change in BMD (9).

Compared to SWAN, which has over 2,200 women, these studies examined a relatively small number of women. In addition, prior studies have not examined women of varying ethnicity. Moreover, all women were postmenopausal so that these studies have not addressed the question of the predictive value of bone turnover in pre- or early postmenopausal women. Finally, the conflicting data from prior studies underscore the need for a large definitive study like ours to determine whether bone turnover markers predict changes in bone density in women. These important issues are being addressed in the SWAN cohort.

2. Use of bone turnover markers to predict changes in bone density in women receiving anti-resorptive therapy.

Several recent studies have examined the ability of bone turnover markers to predict changes in bone density in women who are receiving anti-resorptive therapy with estrogen, raloxifene, or a bisphosphonate. In one study of early postmenopausal women, baseline urinary NTx excretion (but not DPD) and the change in NTx during hormone replacement therapy (HRT) were related to change in spine BMD (1,2). In that study, a 30% decrease in urinary NTx after 6 months of HRT use predicted a 2.2% increase in spine BMD at 1 year and women with the highest baseline levels of NTx, OC, and BSAP levels had the greatest increases in bone density during HRT use (1,2). Of the various bone turnover markers, change in NTx at 6 months was the best predictor of the response to HRT (1). Another study of 569 women found that BSAP, urinary and serum CTx, and serum OC were associated with spine BMD at 2 years (10). A study of 153 early postmenopausal women randomized to HRT or placebo found that after 2 weeks of treatment urinary and serum CTx were associated with BMD at 3 years (11). In contrast, a smaller study failed to find any significant relation between changes in bone turnover markers (free pyridinoline cross links, OC, urinary hydroxyproline, or alkaline phosphatase) and changes in spine BMD in women receiving transdermal estrogen, even though whole body retention of 99mTc-methylene diphosphonate did predict changes in BMD (12). Individual bone turnover markers explained very little of the variability in the change in BMD in women in the PEPI trial who received HRT (9). In early postmenopausal women treated with alendronate for 2 years, baseline serum OC and urinary NTx excretion failed to predict changes in BMD (6). Similarly, baseline serum OC, BSAP, and DPD failed to predict changes in BMD in women over age 65 treated with alendronate although baseline NTx was associated with changes in BMD in these older women (13). In a study of 67 postmenopausal women examining the effect of oral alendronate in prevention of osteoporosis, total OC, serum and urinary CTx, and NTx predicted changes in spine BMD, while BSAP and DPD did not (14). Changes in NTx and OC after 6 months of alendronate therapy were significantly associated with changes in spine and hip BMD in both early and late postmenopausal women (13,15). Even among those studies that report associations between bone turnover measurements and rates of subsequent bone loss, most experts feel that the associations are not strong enough to be clinically useful in individual patients.

3. Use of bone turnover markers to predict osteoporotic fracture risk in postmenopausal women.

In addition to using bone turnover markers to predict changes in BMD with or without anti-resorptive therapy, it is possible that bone turnover markers could help predict the risk of fractures independently from BMD. Cross sectional studies have reported that bone turnover markers (urine pyridinoline, urine CTx, and serum OC) are increased in women with histories of osteoporotic fracture (16,17). The EPIDOS study, a prospective cohort study of 7,598 healthy women over age 75, reported that increased levels of urinary CTx and free DPD (but not urinary NTx) were independent risk factors for hip fracture after adjusting for femoral neck BMD (17). Further, the Hawaii Osteoporosis Study (HOS), a prospective cohort of 512 women derived from the population-based Honolulu Health Program, found that serum BSAP and urinary CTx were associated with spine or nonspine fracture after adjusting for BMD (18). In contrast, the Study of Osteoporotic Fractures (SOF), a prospective cohort study of 9,704 women over age 65, failed to find any relation between serum levels of BSAP, OC, or CTx and the risk of either hip or vertebral fracture (19). An analysis of serum CTx (automated assay method) in the OFELY study did not a correlation with osteoporotic fracture (4). The reasons for the differences between these studies are not known but could be related to the age of the women or the bone turnover markers that were assessed. Changes in serum OC and BSAP and urinary CTx were directly related to the risk of vertebral fracture in women treated with raloxifene (20).

4. Ethnic variation in bone turnover.

One of the major strengths of the SWAN cohort is the ability to examine bone turnover in women of multiple ethnicity (Caucasian, African-American, Chinese, and Japanese). Several recent studies have examined bone turnover in women of differing ethnicity but none has examined a cross-section of women as diverse as in SWAN. For example, Peacock et al reported that serum OC and BSAP levels and urinary free DPD (but not NTx) excretion were lower in elderly African-American women than in Caucasian women (21). Another study compared diurnal patterns of bone resorption in African-American and

Caucasian premenopausal women and found no difference in nighttime urinary free DPD excretion. However, they did report that urinary NTx levels were 25% lower (but not statistically significant) in the African-American women (22). In Japanese women, serum BSAP levels were associated with changes in BMD but accounted for only about 17% of the variation in the change in BMD (23). No other ethnic groups were included in this study. One large study (n=619) investigated geographic variability in bone turnover in postmenopausal women in 10 countries on 4 continents and found that serum OC, serum BSAP, and urine CTx varied significantly by country with the lowest values in Germany and Spain and the highest values in the United States and Canada (24). In SWAN, we will be able to determine if there are differences in bone turnover in premenopausal women that are related to ethnic groups studied and assess whether these relationships change over time as women age and transition through the menopause.

5. Assessment of bone turnover in pre-, peri- and postmenopausal women.

Data comparing bone turnover in pre-, peri- and postmenopausal women are limited. One recent study (OFELY), however, measured bone formation markers (serum OC, BSAP and PINP) and bone resorption markers (urine NTx and CTx) in 653 randomly selected women between the ages of 35 and 89 (25). Of these women 134 were classified as premenopausal (regular menses and FSH level <16.7 IU/L), 42 were classified as early perimenopausal (irregular menses or FSH > 16.7 IU/L, 45 were classified as late perimenopausal (irregular menses and FSH > 16.7 IU/L), and 432 were postmenopausal (no menses for at least 12 months). Ethnicity was not specified though the women were presumably mainly Caucasian. Few significant correlations were found between bone turnover and BMD in premenopausal women though the numbers of perimenopausal women was quite small. BSAP, urine NTx, and urine CTX (but not OC or PINP) were higher in the perimenopausal than in the premenopausal women (25). Another analysis from the OFELY study reported that although there was no change in serum CTx (automated assay) with age, levels were 39% higher in perimenopausal women and 86% higher in postmenopausal women when compared with premenopausal women and that serum CTx was significantly correlated with forearm BMD (4). Finally, a cross-sectional study of a convenience sample of 5,157 women examined the usefulness of urinary NTx in evaluation of bone resorption by age and menopausal status (26). Menopausal status was defined as premenopausal (regular menstrual periods within past 3 months, no changes in frequency and no hot flashes), perimenopausal (menstrual period within 12 months, hot flashes 1-2 times weekly, changes in frequency) and postmenopausal (absence of menstrual period for 1 year and not using hormones. In the age range 45-54 (n=1841) urinary NTx increased from pre- to peri- to postmenopausal status. Further, among perimenopausal women NTx values increased with age.

These interesting findings underscore the need to perform such studies in much larger groups of women and women of multiple ethnicity as in SWAN. Bone turnover markers were recently reviewed in detail (27-29).

B. *Aims and Hypotheses*

This four-year study was a very cost-efficient way to examine longitudinally the relation between bone turnover markers and bone density in mid-aged women as they experience the menopause transition. Building on the multisite Study of Women's Health Across the Nation (SWAN), already funded by the National Institutes of Aging and Nursing Research at the National Institutes of Health, this study assayed already collected and stored specimens of serum to measure bone formation (using an immunoradiometric assay of osteocalcin) and stored urine specimens to measure bone resorption (using urinary N-telopeptide of type I collagen). The analyses in this final report combine the bone turnover data with data from SWAN on bone density (spine, hip and total body), ovarian aging (endogenous sex hormones and menstrual bleeding), and life style factors (exercise, diet, smoking, body mass) to address five research aims. Data are presented for three annual visits, baseline, follow-up 01, and follow-up 02, the three visits for which bone turnover markers were measured under the DOD contract. The aims and hypotheses are as follows:

AIM I: To determine if one-time (baseline) measures of bone turnover markers or changes over time in these measures are associated with the rate of bone loss over a similar time period.
Hypotheses
I.1 Elevated serum osteocalcin levels and/or urinary NTx excretion at baseline will be associated with a greater bone loss ("fast losers") in the subsequent two-year period than normal serum osteocalcin or urinary NTx.
I.2 A persistently elevated serum osteocalcin level or urinary NTx excretion (increased on at least two points in a two-year period will be more strongly associated with greater bone loss over the same period than a single, baseline measure of bone turnover.
I.3. Serum osteocalcin levels and urinary NTx excretion will predict changes in bone density equally well in various racial and ethnic groups.
AIM II: To delineate the longitudinal time course of changes in bone turnover markers both in relation to chronological aging and to changes in menopausal status (ovarian aging).
Hypotheses
II.1. Serum osteocalcin levels and urinary NTx excretion will increase with age but these increases will be associated more closely with changes in menopausal status.
II.2. Serum estradiol will decline and FSH levels will increase prior to the increase in serum osteocalcin levels and urinary NTx excretion.
AIM III: To determine if baseline measures of bone turnover markers are associated with subsequent transition to perimenopause.
Hypotheses
III.1. Elevated baseline serum osteocalcin levels and urinary NTx excretion will predict the transition to perimenopause (i.e. the period of physiologic change associated with menopause) better than baseline levels of FSH and estradiol.
AIM IV: To assess the degree to which potential lifestyle risk factors for osteoporosis (diet, cigarette smoking, exercise, weight) modify the relationships between bone turnover and ovarian aging (Aim I above) and between bone turnover and bone density (Aim II above).
Hypotheses
IV.1 Compared with non-smokers, smokers will have higher baseline serum osteocalcin levels and urinary NTx excretion and stronger associations between these markers and bone loss or ovarian aging, after controlling for chronological aging.
III.2 Body weight will be inversely associated with serum osteocalcin levels and urinary NTx excretion.
AIM V: To characterize bone turnovers in pre- and perimenopausal women belonging to various racial groups and to determine whether observed differences can be accounted for by racial differences in specific lifestyle factors.
Hypotheses
IV.1 At baseline, premenopausal African American women will have higher lower serum osteocalcin levels and urinary NTx excretion than Caucasian or Asian.
IV.2 at baseline, Asian women ill have lower serum osteocalcin levels and urinary NTx excretion than Caucasian women.
IV.3. Time-related increments in serum osteocalcin levels and urinary NTx excretion will be less dramatic in African American women than in Caucasian or Asian women after controlling for menopausal status.
IV4. Racial differences in lifestyle factors such as smoking rates, diet, and body weight will account for part of the difference in baseline serum osteocalcin levels and urinary NTx excretion between African Americans, Caucasians, and Asians.

6. Body

A. Study Objectives

The technical objectives of this four-year project were to:

1. Measure osteocalcin (from serum) and Type I collagen N-telopeptides (from urine) using specimens collected annually at three time points from 2,250 women at five Field Sites across the U.S.; and

2. Combine these data with pertinent data collected concurrently on the same women as part of SWAN to address the Study Aims as delineated in Section B above. The results of analyses are presented in this final report and will be appropriately disseminated as manuscripts are completed by SWAN investigators.

See specific technical objectives listed below under "Completion of Tasks."

B. Study Progress

The study objectives have been completed.

1. Participant Recruitment

Recruitment into the cohort study was completed in December, 1997. Baseline data collection was completed by March, 1998. All recruitment goals for the study were met, resulting in a total of 2,150 participants at the five sites who participated in the bone densitometry and bone marker study. The first annual follow-up began in February 1997 and was completed in January 1999. The second annual follow-up began in March 1998 and data collection was completed at the end of January 2000.

2. Completion of Tasks

Progress on Technical Objectives 1 and 2 are detailed below:

TECHNICAL OBJECTIVE 1:		Measurement and QA/QC of Osteocalcin and Type I collagen N-Telopeptides
Task 1:	Months 1-2:	Finalization of data acquisition protocol

Completion of this task was reported in the October 31, 1997 annual report

Task 2:	Months 1-2:	Finalization of data forms/electronic file formatting

Completion of this task was reported in the October 31, 1997 annual report

Task 3:	Month 3:	Finalization of Manual of Operations

Completion of this task was reported in the October 31, 1997 annual report

Task 4:	Months 2-3:	Design/Testing and implementation of DMS

Completion of this task was reported in the October 31, 1997 annual report

Task 5: Months 4-39: Monthly shipments of specimens to Central Laboratory

Task 6: Months 4-38: Monthly transfer of data results from Central Laboratory to the Coordinating Center

All bone marker assays were completed and the final data file was received by the CC on 09/08/2000. The final data file contained completed results as follows:

	Number of completed assays		
	Baseline	Follow-up 01	Follow-up 02
Osteocalcin	2427	2098	1974
NTx	2428	2100	1974
Creatinine	2428	2100	1974

Task 7: Months 5-39: On-going monitoring of Laboratory performance, including site visits

Throughout the study, the bone marker laboratory maintained quality control procedures that included internal and external quality assurances. Overall, the laboratory maintained a high degree of quality in their laboratory assays and fulfilled the required QC activities.

Task 8: Months 6-42: Assessment of the stability of stored specimens using pooled samples

The SWAN study's Laboratory Oversight Committee is charged with regular review of the laboratory's Standard Operating Procedures and QC data. The LOC has reviewed all SOPs and QC data for assays associated with this study and have found them to meet or exceed all standards.

2. TECHNICAL OBJECTIVE 2: Integrate bone marker data collected under this contract with pertinent data collected concurrently on the same women as part of SWAN. Perform analyses to address the Study Aims as delineated in Section B above. Disseminate results of analyses.

Task 9: Months 15-40: Integration of study and SWAN data into analytic data sets as baseline and follow-up annual data become available

Final, frozen analytic data sets for SWAN baseline bone density (hip and spine), bone markers and all other baseline data are posted on the SWAN internal Web site with appropriate documentation for use by all authorized SWAN investigators. Data cleaning is still in progress for Follow-up 01 bone density and bone markers and for Follow-up 02 bone density and bone markers. It is anticipated that these data will be released in final form by July 2001.

Task 10: Months 18-47: Completion of all analyses.

Analyses to answer each of the above specific aims are presented below. Writing groups are currently working on baseline manuscripts, and completion of these is anticipated by the end of 2001. Manuscripts using longitudinal data will follow shortly after completion of baseline manuscripts.

B. Results

1. Descriptive Statistics

a. Bone Mineral Density, Ethnicity, and Menopausal Status

A total of 2413 participants had bone density measurements at baseline (00). The distribution of participants by ethnic group and SWAN geographic site is shown in Table 1.

Table 1. Number of participants in each ethnic group by site

	SWAN Site					
Ethnic Group	Michigan	Boston	Davis	Los Angeles	Pittsburgh	Total
African American	325	199	-	-	162	686
Chinese	-	-	250	-	-	250
Japanese	-	-	-	281	-	281
White	218	253	209	215	301	1196
Total	543	452	459	496	463	2413

Observed menopausal status categories at baseline and each of the two follow-up visits are displayed in Table 2. By design, at the SWAN baseline visit, all women were either premenopausal, defined as menses in the prior 3 months without any cycle irregularity, or in early perimenopause, defined as menses in the prior 3 months with a change in cycle regularity, and were not using female hormones. For the purpose of these analyses, early and late perimenopause were combined into one category, defined as menses in the prior 3 months with a change in cycle regularity or 3-11 months of amenorrhea. Postmenopause is defined as 12 months of amenorrhea, and surgical menopause as removal of the uterus with or without removal of at least one ovary, or bilateral oophorectomy. Women using hormone replacement therapy (HRT) and those with surgical menopause are placed into a separate category and removed from subsequent analyses as necessary. Once a woman transitioned to a later menopause stage, she could not be classified at an earlier stage in a later follow-up visit.

Table 2. Number of participants in each menopausal status category for each follow-up visit

	Baseline (00)	Follow-up 01	Follow-up 02
1 = Premenopause	1299	556	358
2 = Perimenopause	1094	1429	1315
3 = Postmenopause	-	28	83
4 = Surgical	-	21	48
5 = Hormone user	-	150	231
Missing status	20	229	378

Women were excluded from subsequent data analyses if they reported glucocorticoid use for at least 6 months, use of anticonvulsants or depo Provera for at least 1 year, or if they reported a history of hyperthyroidism, hypercalcemia, chronic liver disease, anorexia nervosa, or bulimia. In addition, data for women who initiated HRT at Follow-ups 01 or 02 were excluded because of the effects of exogenous estrogen on BMD, and women who experienced surgical menopause were excluded because the number of women in this category is too small for meaningful analysis. There were no differences in the percentage of women who were excluded from any of the ethnic groups.

The number of participants in the analysis sample with bone density measurements by anatomic site, menopausal status group, and ethnicity are shown in Tables 3a (baseline), 3b (Follow-up 01), and 3c (Follow-up 02).

Table 3a. Number of bone mineral density (BMD) measurements by ethnicity and menopausal status at baseline

Anatomic Site	Status	African American	Caucasian	Chinese	Japanese	Total
Spine	Pre	317	602	149	161	1229
	Peri	322	519	90	98	1029
Femoral Neck	Pre	327	613	150	163	1253
	Peri	329	536	93	98	1056
Hip	Pre	327	614	150	163	1254
	Peri	329	536	93	98	1056

Table 3b. Number of bone mineral density (BMD) measurements by ethnicity and menopausal status at Follow-up 1

Anatomic Site	Status	African American	Caucasian	Chinese	Japanese	Total
Spine	Pre	117	272	56	48	493
	Peri	306	617	147	167	1237
	Post	9	9	4	5	27
Femoral Neck	Pre	124	280	56	53	513
	Peri	321	644	151	170	1286
	Post	9	9	4	5	27
Hip	Pre	124	280	56	53	513
	Peri	321	644	151	170	1286
	Post	9	9	4	5	27

Table 3c. Number of bone mineral density (BMD) measurements by ethnicity and menopausal status at Follow-up 2

Anatomic Site	Status	African American	Caucasian	Chinese	Japanese	Total
Spine	Pre	66	185	41	36	328
	Peri	301	549	147	157	1154
	Post	31	25	15	6	77
Femoral Neck	Pre	70	188	41	36	335
	Peri	310	572	148	160	1190
	Post	30	25	16	6	77
Hip	Pre	70	188	41	36	412
	Peri	310	572	148	160	1190
	Post	30	25	16	6	77

As shown in Table 4, there were very few observed transitions to postmenopause by Follow-up 02, particularly for the Chinese and Japanese groups. Thus analyses for this final report will focus on changes in BMD and bone markers early in the perimenopausal transition. As discussed in the introduction, most published literature concerns already postmenopausal women. One of the unique contributions of SWAN is the ability to evaluate bone turnover in relation to bone density in women before they reach the final menstrual period.

Table 4. Number of baseline to follow-up 02 bone density measurements by type of menopausal transition and ethnicity

Baseline		PRE	PRE	PERI	PRE	PERI
Follow-up 02		PRE	PERI	PERI	POST	POST
African American						
Spine	N	65	134	166	7	24
Hip	N	70	138	171	7	23
FN	N	70	138	171	7	23
Caucasian						
Spine	N	183	252	288	2	22
Hip	N	188	260	302	2	23
FN	N	188	259	302	2	23
Chinese						
Spine	N	41	85	59	5	10
Hip	N	41	85	61	5	11
FN	N	41	85	61	5	11
Japanese						
Spine	N	36	88	64	-	6
Hip	N	36	93	64	-	6
FN	N	36	93	64	-	6

FN = Femoral Neck

Table 5 displays descriptive statistics for bone mineral density and bone markers, and for the other variables used as predictors in the subsequent analyses: age, weight, body mass index (BMI), and levels of follicle stimulating hormone (FSH) and estradiol (E2).

Table 5. Descriptive Statistics for outcome and predictor variables in the analysis sample

	N	Min	Max	Median	Mean	S.D.
Bone mineral density						
Spine						
Baseline	2277	0.720	1.636	1.075	1.078	0.139
Follow-up 01	1760	0.699	1.620	1.070	1.074	0.140
Follow-up 02	1617	0.658	1.626	1.061	1.069	0.143
Hip						
Baseline	2330	0.540	1.540	0.953	0.964	0.146
Follow-up 01	1830	0.530	1.557	0.949	0.960	0.146
Follow-up 02	1662	0.588	1.540	0.945	0.956	0.146
Femoral Neck						
Baseline	2329	0.495	1.470	0.836	0.846	0.135
Follow-up 01	1830	0.481	1.549	0.824	0.839	0.135
Follow-up 02	1662	0.498	1.527	0.821	0.856	0.134
Hormone Levels						
FSH						
Baseline	2364	1.11	273.80	15.8	23.74	24.9
Follow-up 01	1889	1.30	268.00	18.4	30.39	33.3
Follow-up 02	1689	0.39	394.20	21.3	40.94	46.2
Estradiol						
Baseline	2248	5.40	1493.55	55.2	77.29	84.9
Follow-up 01	1745	5.05	849.50	48.0	72.29	77.8
Follow-up 02	1689	4.25	6987.20	38.0	69.85	187.3
Bone Turnover Markers						
Osteocalcin						
Baseline	2355	2.9	91.3	15.2	16.04	6.09
Follow-up 01	1889	2.1	71.2	15.1	16.35	6.64
Follow-up 02	1683	3.1	85.7	16.2	17.56	7.62
NTx adjusted for creatinine						
Baseline	2391	5.2	272.7	30.5	34.88	20.1

Follow-up 01	1926	5.1	345.3	30.2	35.19	21.8
Follow-up 02	1711	4.2	416.8	32.2	37.25	22.8
Other Variables						
Age (years)						
Baseline	2413	42.0	53.0	46.2	46.3	2.67
Follow-up 01	2242	42.8	54.6	47.1	47.3	2.66
Follow-up 02	2069	43.9	55.2	48.1	48.3	2.62
Weight (kg.)						
Baseline	2395	37.6	153.9	69.4	74.0	20.7
Follow-up 01	1950	39.0	181.3	69.0	73.7	20.9
Follow-up 02	1772	38.7	179.7	69.0	74.2	21.0
BMI (kg/cm^2)						
Baseline	2373	14.99	56.99	25.99	27.91	7.31
Follow-up 01	1950	14.33	62.07	25.79	27.86	7.35
Follow-up 02	1771	16.61	66.34	26.01	28.12	7.43

Boxplots of the distribution of baseline BMD for each of the three anatomic sites by ethnicity and by menopausal status are presented in Figure 1. The rectangle represents the interquartile range (25% to 75% percentiles) of the data and the horizontal line in the middle of this rectangle depicts the median value. The vertical lines represent the dispersion of the data, and the horizontal marks outside the vertical lines represent extreme data values. The highest BMD at all anatomic sites was observed in both pre- and early perimenopausal African American women ($p<0.0001$ from analysis of variance), and the lowest in Chinese and Japanese women, who were similar to each other. Baseline BMD of the spine, femoral neck, and total hip were all higher in Caucasian women than in Chinese or Japanese women. Differences by menopause status within ethnicity were smaller than ethnic differences in BMD. Results were similar for follow-ups 01 and 02.

Figure 1. Distribution of baseline bone mineral density by ethnicity and menopause status for the spine, hip, and femoral neck anatomic sites

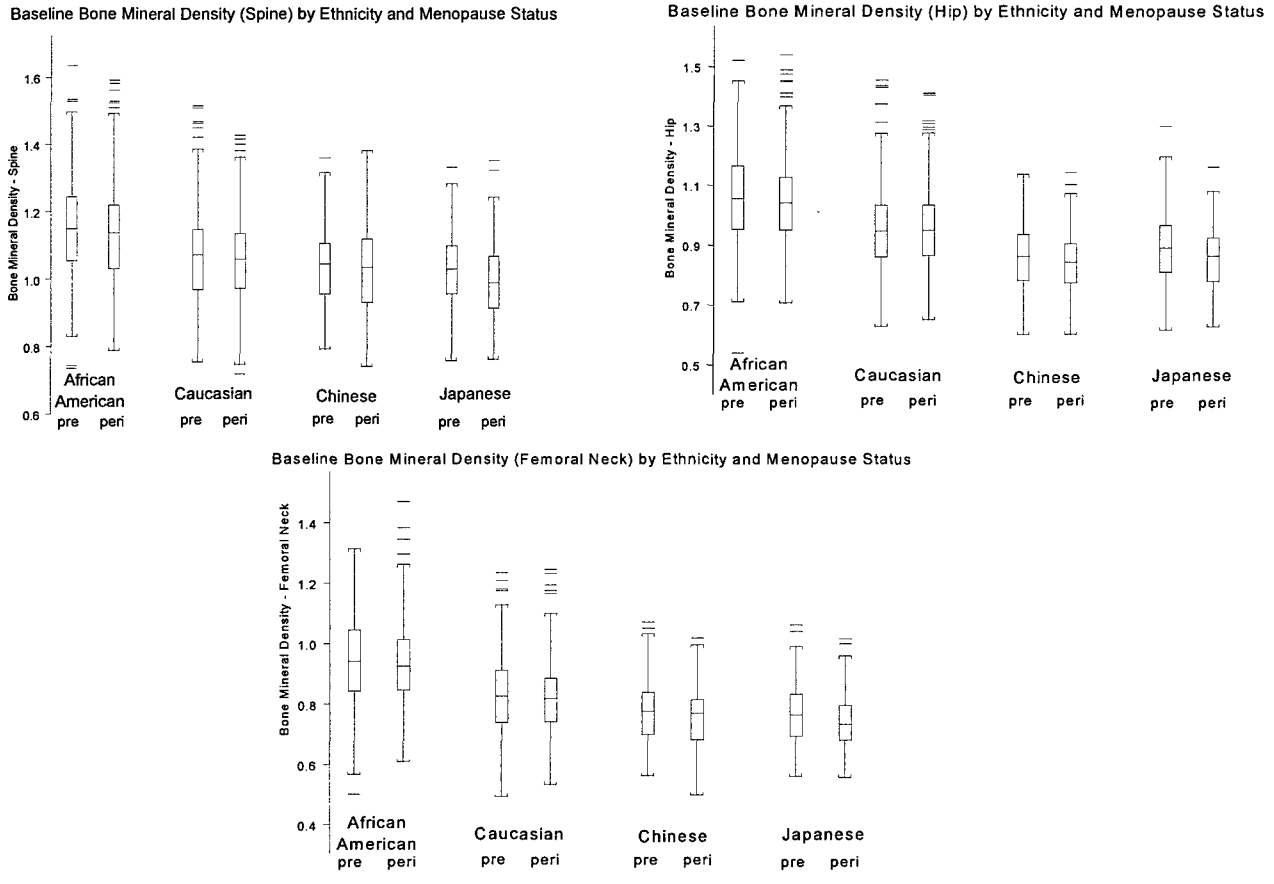
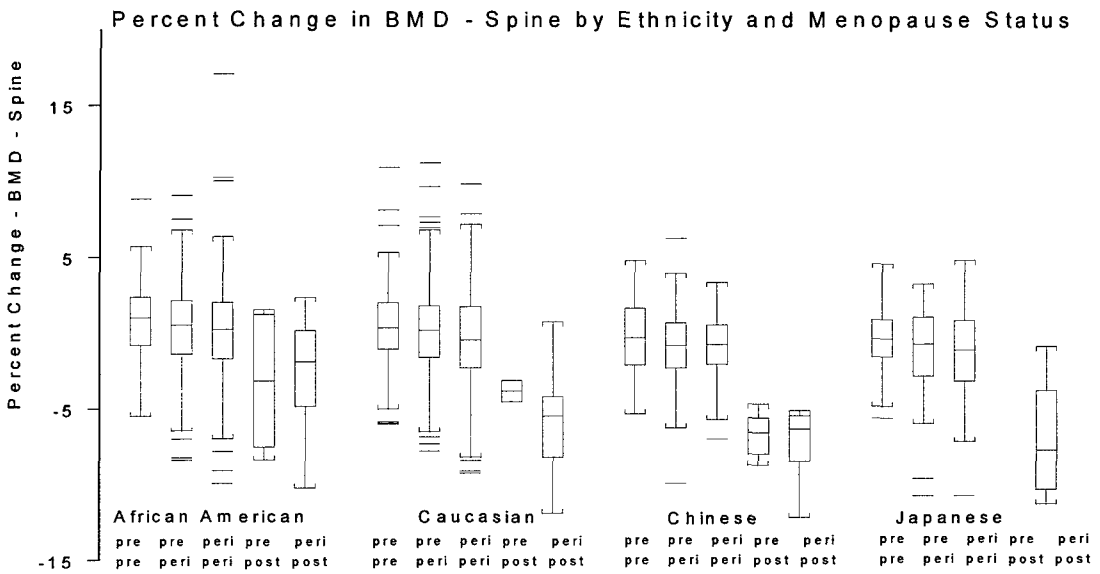
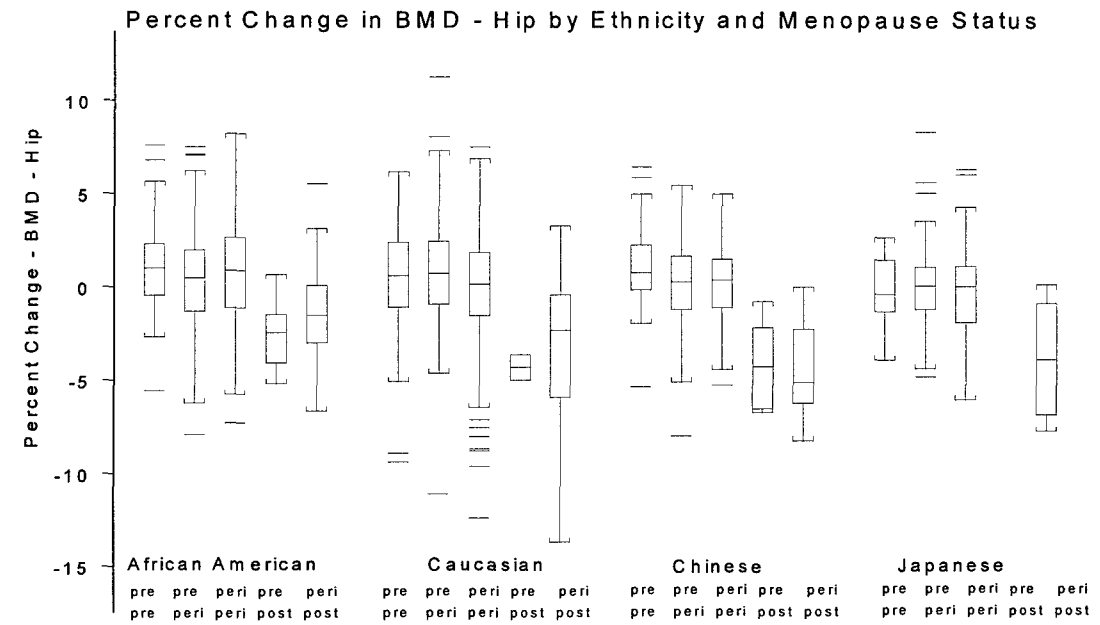
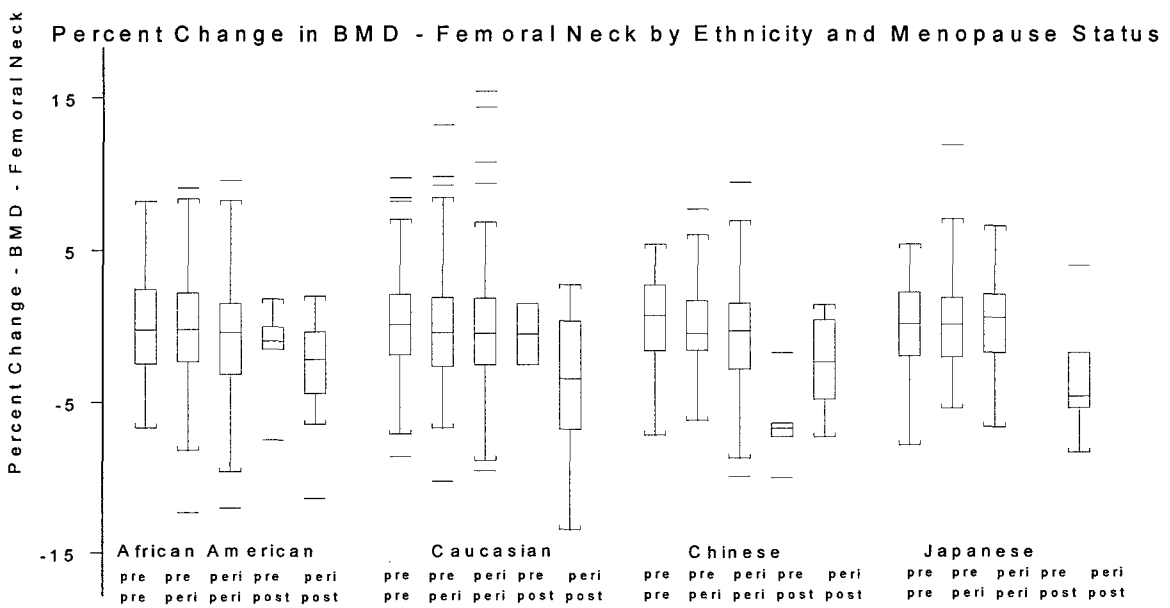


Figure 2 displays the percent change in mean BMD from baseline to the second annual follow-up. For each anatomic site, the percent change in BMD is shown by ethnic group and by change in menopausal status within each ethnic group. Overall, mean BMD decreased from baseline to Follow-up 02, particularly for women who transitioned from pre-to postmenopause or from peri to postmenopause, although the sample size for these categories is very small (see Table 4).

Figure 2. Percent change in BMD – from Baseline to 02 by ethnicity and menopause status







b. Descriptive statistics for bone turnover markers

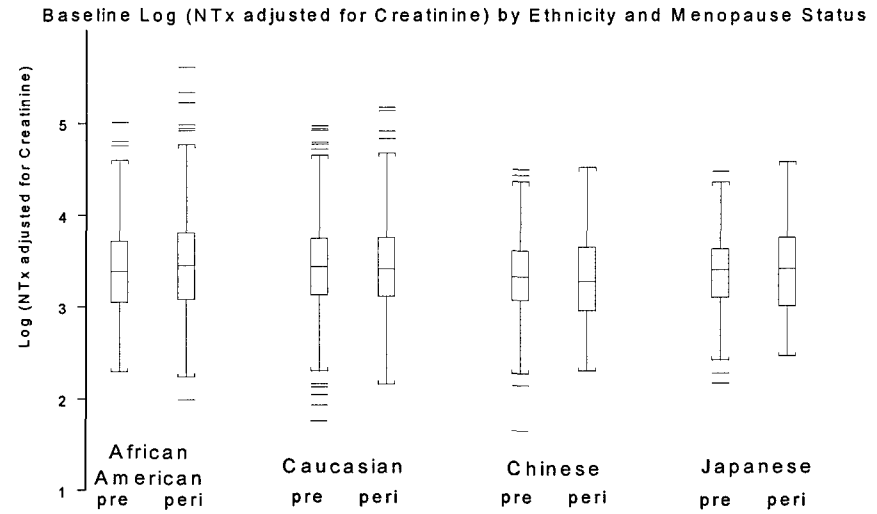
The number of bone turnover assays for each visit by ethnic group is shown in Table 6.

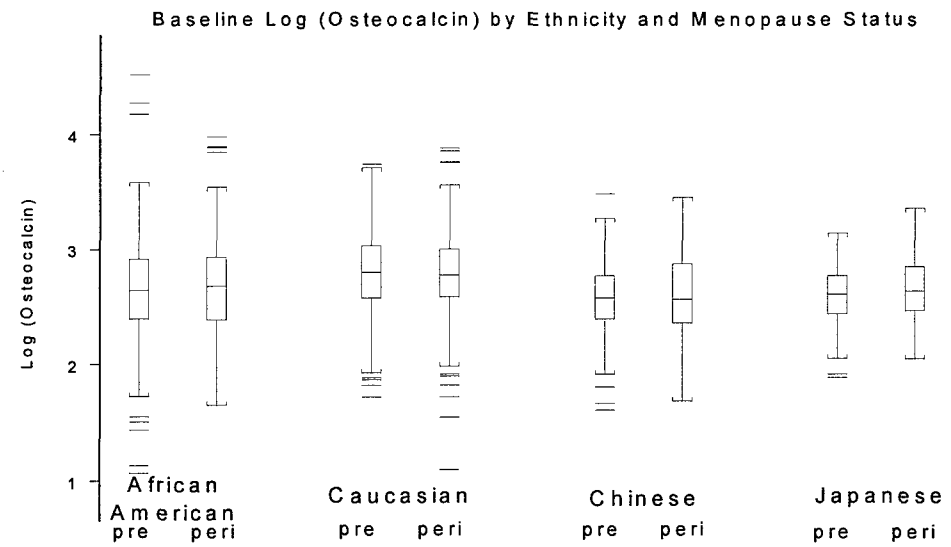
Table 6. Number of bone turnover measurements by visit and ethnicity in the analysis sample.

Visit	Marker	African American	Caucasian	Chinese	Japanese	Total
Baseline	NTx adjusted	680	1184	248	279	2391
	Osteocalcin	653	1176	248	278	2355
Follow-up 01	NTx adjusted	520	1076	238	262	2096
	Osteocalcin	504	1050	235	260	2049
Follow-up 02	NTx adjusted	487	993	231	255	1966
	Osteocalcin	482	974	224	255	1935

The distributions of osteocalcin and NTx (adjusted for creatinine) at baseline are shown in Figure 3 by ethnicity and menopausal status. Because of skewed distributions, values of both bone markers were log-transformed for analyses. There was little variation in unadjusted bone turnover marker levels by ethnic group and by menopause status at baseline.

Figure 3. Baseline bone markers by ethnicity and menopause status





The information in Table 7 below shows that unadjusted mean levels of osteocalcin and NTx increase as women progress to a later stage of menopause. This trend is similar for all ethnic groups. Changes in mean bone turnover markers from baseline to follow-up 02 are also positively associated with baseline age and with longer time since baseline.

Table 7. Descriptive statistics for bone turnover markers by ethnicity, time, and menopause status

		PRE			PERI			POST		
Var.	Visit	N	Mean	SD	N	Mean	SD	N	Mean	SD
African American										
NTx	00	340	3.40	0.488	338	3.48	0.558	-	-	-
	01	128	3.28	0.448	337	3.45	0.548	9	3.76	0.629
	02	68	3.33	0.455	314	3.42	0.526	31	3.84	0.595
Ost	00	324	2.64	0.429	327	2.67	0.421	-	-	-
	01	123	2.59	0.390	329	2.65	0.457	9	3.17	0.541
	02	67	2.59	0.444	309	2.67	0.476	31	3.01	0.490
Caucasian										
NTx	00	624	3.44	0.460	548	3.45	0.493	-	-	-
	01	288	3.43	0.501	669	3.46	0.497	10	4.05	0.436
	02	180	3.43	0.390	582	3.53	0.46	24	3.96	0.386
Ost	00	628	2.80	0.316	536	2.78	0.320	-	-	-
	01	281	2.75	0.330	654	2.82	0.356	10	3.23	0.529
	02	177	2.79	0.352	570	2.87	0.350	24	3.25	0.412
Chinese										
NTx	00	153	3.33	0.477	93	3.29	0.526	-	-	-
	01	59	3.17	0.397	164	3.35	0.501	4	4.27	0.93
	02	40	3.40	0.323	154	3.51	0.418	18	4.07	0.432
Ost	00	153	2.57	0.300	93	2.60	0.362	-	-	-
	01	59	2.51	0.318	162	2.64	0.341	4	3.18	0.313
	02	40	2.59	0.265	148	2.71	0.344	17	3.26	0.319
Japanese										
NTx	00	170	3.39	0.424	105	3.39	0.424	-	-	-
	01	59	3.27	9.448	184	3.43	0.455	5	3.85	0.206
	02	40	3.38	0.336	179	3.52	0.457	6	4.01	0.712
Ost	00	170	2.59	0.247	104	2.65	0.272	-	-	-
	01	57	2.57	0.269	185	2.65	0.307	5	3.128	0.364
	02	40	2.62	0.328	179	2.75	0.358	6	3.30	0.408

NTx = log (NTx adjusted for creatinine) Ost = log (Osteocalcin)
N = sample size SD = standard deviation

3. Analyses for the Specific Aims

AIM I: *To determine if one-time (baseline) measures of bone turnover markers or changes over time in these measures are associated with the rate of bone loss over a similar time period.*

Do elevated levels of bone turnover markers at baseline predict greater bone loss in the subsequent two-year period than normal levels? Are elevated marker levels at two or more time points more strongly associated with greater bone loss than a single, baseline measure?

We fit least squares regression models with an outcome of percent change in bone mineral density (BMD) from baseline to visit 2 and predictors log osteocalcin and log NTX at visit i (i=baseline, one year later, and two years later), adjusted for age at baseline and years from baseline to visit 2. The data set is "trimmed" to exclude a few extreme BMD changes to make model residuals more closely approximate a normal distribution. The first set of models includes only baseline bone turnover markers, the second set adds visit 1 markers to baseline markers, and the third adds visit 2 markers to prior markers.

Baseline NTx is inversely associated with change in total spine and total hip BMD (higher baseline NTx is associated with greater bone loss), while baseline osteocalcin is not associated with later change in

BMD. Neither bone marker is associated with change in BMD at the femoral neck of the hip. However, later bone marker measures at follow-ups 01 and 02 add dramatically increase the model's explanatory power regarding change in BMD. R^2 (percent of total variation explained by model predictors and covariates) increases as follows (Table 8):

Table 8. Percentage of variability in percent BMD change explained by model predictors and covariates

	Baseline markers	Baseline plus visit 1 markers	Markers at all 3 visits
Total spine	4.8%	11.0%	18.8%
Total hip	3.0%	8.9%	13.6%
Femoral neck	1.2%	3.9%	5.6%

While baseline NTx is somewhat predictive of two-year change in total spine and hip BMD, knowledge of both bone markers at visit 1 adds significantly to this predictive power, while adding visit 2 bone markers further explains change in BMD. Both markers contribute independently toward explaining change in BMD for models, which include visit 1 or visit 2.

Is there a difference among ethnic groups in the predictive ability of bone turnover markers?

Finally, we checked whether the relation between bone marker and BMD change differs by ethnicity by including ethnic interaction terms in the models described above. Only for total spine did we find such an interaction- higher osteocalcin at visit 2 for African-Americans (and to a lesser extent for Caucasians) was associated with a smaller amount of bone loss than for Asians (p=.0006). That is, while higher osteocalcin is associated with more bone loss in all ethnic groups, for a given increase in osteocalcin one would expect more loss among Asian women.

To confirm these results from parametric methods, we also used generalized additive models, a nonparametric technique, to examine the influence of ethnic group on the association of BMD loss from baseline to 02 and adjusted NTx and osteocalcin at time 2. Bone loss was calculated as: Loss = (BMD Time 2 – BMD Time 0)*100/BMD Time 0.

No ethnic differences were detected at the hip and femoral neck sites with regard to the relation between osteocalcin and bone loss or the relation between NTx and bone loss. African Americans and Caucasians differed from Chinese and Japanese women, but were similar to each other for both markers at the spine site.

In conclusion, it appears that there is minimal predictive value of baseline bone markers for subsequent two-year bone loss. Association of bone turnover with bone loss becomes stronger with proximity to the end of the two-year observation period.

AIM II: *To delineate the longitudinal time course of changes in bone turnover markers both in relation to chronological aging and to changes in menopausal status (ovarian aging).*

Changes in bone markers with age and advancing menopausal status

Osteocalcin and NTx changes from baseline to visit 2 are positively associated with baseline age and with longer time since baseline. Changes in both markers are also positively associated with change to more advanced menopausal status (see Table 7).

Results below are from ordinary least squares regression models with change in bone marker level from baseline to visit 2 as the dependent variable. All models adjust for baseline bone marker level. Separate models were fitted for baseline age plus time since baseline, and for menopausal status. The column

labeled Model 4 presents results for all these variables in a single model, to determine whether they are independently associated with changes in bone markers.

For osteocalcin, independently of menopause status, a larger increase is expected from baseline to visit 2 for an older woman and for a woman whose elapsed time between visits is greater (regardless of baseline age) (Table 9). Independently of baseline age and time since baseline, women who are pre-menopausal at both baseline and visit 2 have a negligible (non-statistically significant) mean increase in osteocalcin, while women who transition to perimenopause have a larger (significant) mean increase, women who remain perimenopausal have a still larger mean increase, and women who transition to postmenopause have the largest mean increase. The estimates for the transition to postmenopause are less precise due to few post-menopausal women at visit 2. All three factors, age, time elapsed, and menopause status contribute independently to an osteocalcin increase (see Model 4, Table 9).

Table 9. Linear regression models for change in serum osteocalcin from baseline to follow-up 02 adjusted for baseline osteocalcin (N=1557)

	Model 1	Model 2	Model 3	Model 4
Slope coefficient (SE) for baseline age	0.40 (0.05)****			0.29 (0.05)****
Slope coefficient (SE) for years since baseline		3.20 (0.99)***		3.10(0.97)***
Mean osteocalcin (SE) by status transition:				
Pre-pre (n=314)			.06 (.29)	.38 (.29)
Pre-peri (n=586)			1.28 (.21)	1.27 (.21)
Peri-peri (n=584)			1.86 (.21)	1.81 (.21)
Pre-post (n=11)			5.26 (1.57)	4.76 (1.55)
Peri-post (n=62)			7.29 (.67)	6.48 (.67)
P-value for overall status difference			<0.0001	<0.0001
P-value for pre-pre vs. pre-peri			0.0008	0.015

* p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001

Table 10 displays corresponding results for urinary NTx adjusted for creatinine. Results for NTx are similar to those of osteocalcin, but associations are weaker. Higher age at baseline is associated with a larger mean NTx increase, but time since baseline is not statistically significant. Advanced status is in general associated with higher mean turnover increase, but the pre-pre versus pre-peri difference is not statistically significant. These results suggest that urinary NTx levels increase at a later point in the menopausal transition than do osteocalcin levels.

Table 10. Linear regression models for change in NTx from baseline to follow-up 02 adjusted for baseline NTx (N=1605)

	Model 1	Model 2	Model 3	Model 4
Slope coefficient (SE) for baseline age	1.06 (0.19)****			0.71 (0.20)***
Slope coefficient (SE) for years since baseline		4.21 (3.74)		4.03 (3.69)
Mean osteocalcin (SE) by status transition:				
Pre-pre (n=322)			-1.56 (1.13)	-0.81 (1.15)
Pre-peri (n=592)			1.09 (0.84)	1.06 (0.83)
Peri-peri (n=614)			4.77 (0/82)	4.62 (0.82)
Pre-post (n=14)			20.34 (5.43)	18.88 (5.43)
Peri-post (n=63)			16.95 (2.59)	15.05 (2.64)
P-value for overall status difference			< 0.0001	< 0.0001
P-value for pre-pre vs. pre-peri			0.06	0.19

* p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001

Does change in serum hormones (estradiol and FSH) precede increasing bone turnover?

We examined change in hormones from baseline to follow-up 01, compared to change in bone markers from follow-ups 01 to 02.

In general, osteocalcin and NTx are positively correlated at all 3 visits (Pearson correlation is approximately .50), while FSH and estradiol (E2) are negatively correlated (Pearson correlation is approximately -.45). FSH is positively correlated with both bone markers (Pearson correlations between .09 and .27 at various visits), while E2 is weakly correlated with NTx (Pearson correlation from -.06 to -.15) and uncorrelated with osteocalcin.

Changes in hormones preceding changes in bone markers were examined in three distinct ways. The first forms a 2 by 2 table of above/below median bone marker change from follow-up 01 to follow-up 02 cross-classified by above/below median hormone change from baseline to follow-up 01. Fisher's exact test was used to assess statistical significance. The second is a 4 by 4 table using quartiles of change for the two variables. The Mantel test was used to assess linear trend. The final approach is to model the change in bone marker from follow-ups 01 to 02 as a function of 01 bone marker level, baseline hormone level, change in hormone level from baseline to 01, baseline age, and time from baseline to 02. The effect of baseline to 02 change in menopause status was also evaluated, but, attrition due to surgical menopause, or initiating HRT after baseline reduced the sample size of such models by about 260 participants. Models were run with all the data, and with a "trimmed" subset of the data omitting extreme bone marker changes. Log versions were also run for NTx.

Results for E2: The change in serum E2 level from baseline to follow-up 01 appears unrelated to change in either bone marker from 01 to 02, using all three types of analyses. Nothing is close to statistical significance except for a linear trend test of E2 change by osteocalcin change using change quartiles (p=.06). 27% compared to a null-hypothesized 25% of the observations are inversely concordant in the 4 by 4 table (inverse in that low quartile of one is matched with high quartile of the other, thus 27% of the observations are on the inverse diagonal of the table).

Results for FSH: Change in FSH level from baseline to follow-up 01 has a fairly strong relation to change in osteocalcin level from 01 to 02. Without adjustment, 825 of 1536 (53.7%) change observations are concordant in the 2 by 2 table formed by median splits of changes in osteocalcin and FSH (p=.004). In the 4 by 4 table of change quartiles, 30% compared to a null-hypothesized 25% of the observations are fully concordant (p=.003 for the linear trend test). After adjusting for covariates noted above (except for menopause status), an estimated increase of .023 mIU/mL in FSH is associated with a 1 ng/mL increase in osteocalcin (SE=.0043, p<.0001). This adjusted relation changes little if trimmed data are used, or if status combination is added to the models. Results are consistent using all three methods.

Change in FSH from baseline to follow-up 01 appears unrelated to change in NTx from 01 to 02 using two of the methods, but the third suggests a possible relation. The median split and quartile split methods, unadjusted for covariates, show no relation between the two (p=1.00 and .89 respectively). A simple Pearson correlation between the two is also non-significant (p=.84). However, after adjusting for covariates, and in particular for follow-up 01 NTx level, the relation becomes statistically significant. The estimated coefficient for change in NTx is .083 nM BCE per liter per mM creatinine per liter (SE=.015, p<.0001). However, the relation is somewhat less significant using trimmed data (est. coefficient=.044, SE=.013, p=.0005), still less using logged data (and differences of logs; p=.009), and non-significant using trimmed or logged data with status transition added (p=.08 and .26 respectively).

Thus, change in FSH is unrelated to change in NTx without considering other variables. But it appears that if one knows the value of NTx at follow-up 01, an increase in FSH from baseline to follow-up 01 may be associated with an increase in NTx from follow-up 01 to 02. Change in FSH by itself does not help predict a later change in NTx, but if one also knows current NTx, knowing the prior year's change in FSH may help. However, since not all regression models agree, this result is tentative at best. Additional follow-up data would be needed to form definitive conclusions.

In conclusion, a change in E2 level early in the menopausal transition is not a good predictor of a future change in serum osteocalcin or urinary NTx, while change in FSH over the past year does help predict change in osteocalcin over the next year, and may, in conjunction with current NTx, help predict future change in NTx.

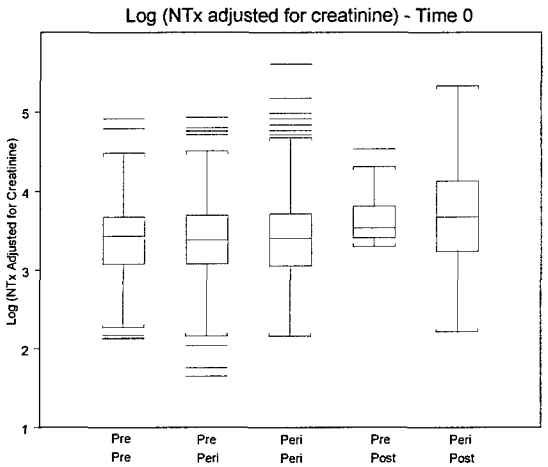
AIM III: *To determine if baseline measures of bone turnover markers are associated with subsequent transition to perimenopause.*

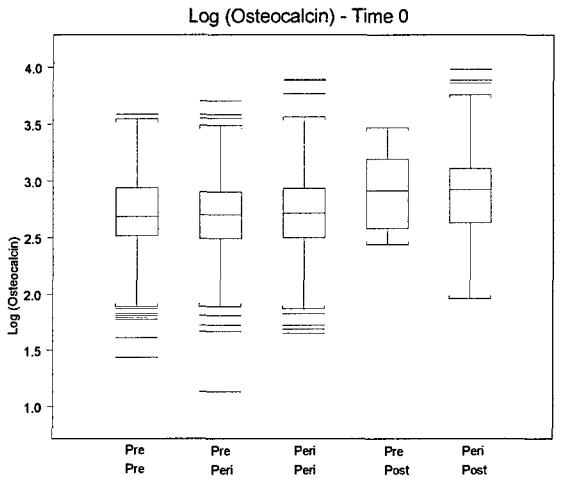
Do baseline bone markers predict the transition to perimenopause?

Figure 4 presents box plots of the distribution of baseline log NTx and osteocalcin for each of the menopause status transition categories (1=pre/pre 2=pre/peri 3=peri/peri 4=pre/post 5=peri/post). P-values for comparing transition categories were calculated from a one-way analysis of variance. Multiple comparisons use the Tukey's studentized range.

Overall, baseline log NTx varied significantly by menopause transition (p<0.0001). Mean log NTx was significantly higher for the peri-post transition than for pre-pre, pre-peri, and peri-peri. Pre-pre and pre-peri, however, did not differ significantly. A similar pattern was noted for baseline serum osteocalcin. Thus, changes in bone markers seem to occur later in the transition, and do not appear to be strongly associated with the transition from pre- to perimenopause.

Figure 4. Distribution of NTx and osteocalcin at baseline by menopause status





Using analysis of covariance for the subset of women who were premenopausal at baseline, we could find no evidence that baseline levels of osteocalcin and NTx predict subsequent transition to peri-menopause in approximately two years (Table 11). Results are reported on natural log and original scales; natural log results better meet the assumption of normally distributed data. Some evidence does exist that later transition to post-menopause is associated with quite high baseline bone markers, although very few pre-menopausal women are post-menopausal two years later:

Table 11. Adjusted* means and std. errors (SE) for baseline levels of osteocalcin and NTx by follow-up 02 menopause status for women pre-menopausal at baseline

		Natural log scale		Original scale	
Follow-up 02 menopause status	Number of women	Osteocalcin (SE)		Osteocalcin	NTx
PRE	325	2.71 (.019)	3.40 (.027)	15.9 (.29)	33.6 (.96)
PERI	590	2.69 (.014)	3.39 (.020)	15.5 (.22)	33.2 (.70)
POST	12	2.93 (.097)	3.72 (.136)	19.7 (1.51)	44.3 (4.94)
PRE vs. PERI p-value		.45	.76	.30	.71
P-value comparing all 3 groups		.047	.057	.018	.08

* adjusted for current smoking and age at baseline.

Do bone markers predict the transition to perimenopause better than baseline levels of FSH and E2?

Higher levels of baseline follicle-stimulating hormone (FSH) are associated with later transition to peri-menopause, while baseline estradiol (E2) is not significantly related to subsequent transition (Table 12). As expected, highest baseline FSH levels are found in the 12 women who are post-menopausal after two years:

Table 12. Means and std. errors (SE) for baseline levels of FSH and E2 by menopause status at follow-up 02 for women pre-menopausal at baseline adjusted for baseline current smoking and age

		Natural log scale		Original scale	
Follow-up 02 menopause status	Number of women	FSH (SE)	E2 (SE)	FSH	E2
PRE	325	2.62 (.031)	4.10 (.039)	15.9 (.77)	76.2 (4.81)
PERI	590	2.79 (.023)	4.01 (.029)	19.6 (.57)	74.7 (3.54)
POST	12	3.62 (.162)	3.78 (.203)	52.0 (5.17)	48.5 (24.7)
PRE vs. PERI p-value		<.0001	.08	<.0001	.80
P-value comparing all 3 groups		<.0001	.10	<.0001	.54

Baseline bone markers and hormones and subsequent transition

In the subset of women who were premenopausal at baseline, we next fitted logistic regression models with menopause status (pre and peri only) at time 2 as the outcome, using bone markers, E2 and FSH as predictor variables, adjusting for baseline age and current smoking status. Putting both hormones in the same model, FSH was predictive of subsequent transition to peri-menopause (p=.0002; 95% confidence interval on log scale (1.32, 2.39)), while E2 was not (p=.87). Neither bone marker was predictive of subsequent menopause status, either separately or together. In a model with all four hormones and markers together, only FSH was associated with subsequent transition (p=.0001; 95% CI (1.33, 2.41)). Thus, of the 4 potential predictors, only FSH at baseline helps predict subsequent transition to peri-menopause.

The number of women who transitioned from pre-to postmenopause was too small (n=12) for stable regression modeling, thus these women were omitted from the multivariate models.

In conclusion, baseline bone turnover markers were not good predictors in premenopausal women of the transition to perimenopause. The only significant predictor of entry into the perimenopause in the above models was baseline FSH level.

AIM IV: *To assess the degree to which potential lifestyle risk factors for osteoporosis (diet, cigarette smoking, exercise, weight) modify the relationships between bone turnover and ovarian aging (Aim I above) and between bone turnover and bone density (Aim II above).*

Effects of baseline smoking, calcium intake, weight, and weight change on relation between bone markers and change in bone mineral density

Table 13 displays the distribution of smokers by ethnicity in the analysis sample.

Table 13. Current Smoking by ethnic group and visit, n=1743

Current Smoker at Baseline	Visit	African American	Caucasian	Chinese	Japanese
No	Baseline	346	723	212	198
Yes		112	109	1	30
No	Follow-up 01	313	713	210	199
Yes		91	100	1	26
No	Follow-up 02	345	731	211	204
Yes		109	99	2	25

Current smoking at baseline is not associated with baseline NTx (p=.33 for t-test), while smokers have **lower** baseline osteocalcin (means for smokers and non-smokers are 14.85 and 16.22, p=.001). Baseline weight is mildly correlated with baseline bone markers. Pearson correlation coefficients with log NTx and log osteocalcin are -.06 and -.10.

Smoking, calcium, weight, and weight change were added to the models described in Aim I. Results are as follows:

For change in total spine BMD, adding these covariates to the model with bone markers at all 3 visits increases the R^2 from 18.8% to 19.7%, without materially changing associations between bone markers and change in spine BMD. The interaction between osteocalcin and ethnicity (see Aim 1) is still significant (p=.0007) with added covariates, and in the same direction. Only baseline weight is statistically significant (p=.03) among the added covariates.

For change in femoral neck, the R^2 increases from 5.6% (Aim1 without covariates) to 10.6% with covariates. Higher weight increase is strongly associated with higher bone loss. This covariate mutes the association between bone markers and change in BMD, but osteocalcin in particular remains significantly associated (inversely) with change in BMD.

Change in weight is also the most significant added covariate when modeling change in total hip BMD (direct association). R^2 increases from 13.6% to 22.5% with covariates. Both NTx and osteocalcin remain independently associated (inversely) with change in hip BMD, although associations are again diminished when weight change is considered.

Effects of smoking, calcium intake, and weight on bone marker changes, which occur with advancing age and menopausal status (extension of Aim 2)

This section considers whether current smoking at baseline, intake of calcium from diet and supplements, and baseline weight plus change in weight since baseline alter the relation between change in bone markers and age and menopausal status.

Several different models were used to answer the general question. One set of models includes five menopausal transition combinations and all the available data. Because change in bone marker level is somewhat right-skewed, trimmed data omitting some large outliers was also used in modeling. Full and trimmed data versions were also used for data with only two transition combinations (pre to pre, pre to peri-menopause). Finally, for change in NTx (which is particularly skewed), log transformed versions were run. General conclusions were unaffected by transformations or trimming, so untransformed, untrimmed results are reported.

Possible interactions with smoking, calcium, and weight were considered for all models. Only statistically significant interactions were retained in final models.

Parameter estimates (slopes or means) for effects of interest are shown below in Table 14, along with standard errors and significance levels under the null hypothesis, for models including smoking, calcium, and weight and significant interactions between them and age or status, and for models omitting smoking, calcium, and weight (that is, unadjusted for these covariates).

Table 14. Linear regression models for two-year change in osteocalcin, without and with adjustment for smoking, calcium, and weight

	Unadjusted model	Adjusted model
	N=1557	N=1514
Slope (SE) for baseline age	0.29 (0.05)****	0.29(0.05)****
Slope (SE) for years since baseline	3.10 (0.97)***	2.43 (0.99)*
Means (SE) by status transition		
Pre-pre (n=311)	0.38 (0.29)	0.36 (0.29)
Pre-peri (n=557)	1.27 (0.21)	1.22 (0.21)
Peri-peri (n=561)	1.81 (0.21)	1.90 (0.22)
Pre-post (n=11)	4.76 (1.55)	4.58 (1.54)
Peri-post (n=61)	6.48 (0.67)	6.57 (0.67)
P-value for overall transition differences	< 0.0001	< 0.0001
P-value for pre-pre vs. pre-peri	0.015	0.017
Slope (SE) for:		
Baseline smoking		-0.48 (-.40)
Calcium (gm)		0.14 (0.23)
Baseline weight (kg)		-0.36 (0.0065)****
Change in weight		-0.109 (0.028)****

* p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001

The added covariates do not materially alter the original relation between change in osteocalcin, and age and transition combination. Baseline age, time since baseline, and status are still all independently associated with change in osteocalcin. Whether or not one adjusts for the added covariates, those who made the transition from pre to peri-menopause had about a .9 ng/mL greater increase in osteocalcin than those who remained pre-menopausal over the two-year period. There were no statistically significant interactions between the added covariates and age or menopause status. Among the added covariates, only baseline weight and change in weight are associated, inversely, with change in osteocalcin.

Use of trimmed data does not change these conclusions. Using trimmed data, however, there is a statistically significant interaction between baseline age and weight (p=.02 for a model with trimmed data and all 5 statuses). If age is held constant and weight increases, osteocalcin declines, but more so if a woman is older. Similarly, if weight is held constant and age increases, osteocalcin increases, but less so if the woman is heavier. This relation also holds in a model using trimmed data with only pre-pre and pre-peri women (p=.002).

Table 15. Linear regression models for two-year change in NTx, without and with adjustment for smoking, calcium, and weight

	Unadjusted model	Adjusted model
Slope (SE) for baseline age	0.71 (0.20)***	2.34 (0.58)****
Slope (SE) for years since baseline	4.03 (3.69)	4.56 (3.03)
Means (SE) by status transition		
Pre-pre (n=319)	-0.64 (0.91)	-0.81 (1.15)
Pre-peri (n=576)	1.17 (0.67)	1.06 (0.83)
Peri-peri (n=586)	4.11 (0.66)	4.62 (0.82)
Pre-post (n=12)	10.06 (5.83)	18.88 (5.43)
Peri-post (n=60)	12.14 (2.13)	15.05 (2.64)
P-value for overall transition differences	< 0.0001	< 0.0001
P-value for pre-pre vs. pre-peri	0.11	0.19
Slope (SE) for:		
Baseline smoking		-0.96 (1.21)
Calcium (gm)		10.10 (3.13)***
Baseline weight (kg)		0.89 (0.35)*
Change in weight		-0.38 (0.87)****

* p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001

These results are somewhat more complicated than for osteocalcin, because of the detection of age by weight, and calcium by status, interactions (not presented in Table 15). But the basic conclusions are the same as in the unadjusted model. Higher age at baseline is still associated with increased NTx compared to lower age; time since baseline and pre-pre versus pre-peri status categories are still not significant. The age/weight interaction has the same interpretation as for osteocalcin.

In conclusion, smoking seems to have no effect on the relation between bone marker change and age, time since baseline, and status combination. Calcium intake has little effect except for a possible interaction with status for change in NTx (post-post group differs from others), while weight seems to interact somewhat with age, and possibly with status for NTx. Baseline weight and change in weight since baseline both appear inversely related to change in markers.

Does smoking alter the relation between change in hormones and increasing bone turnover?

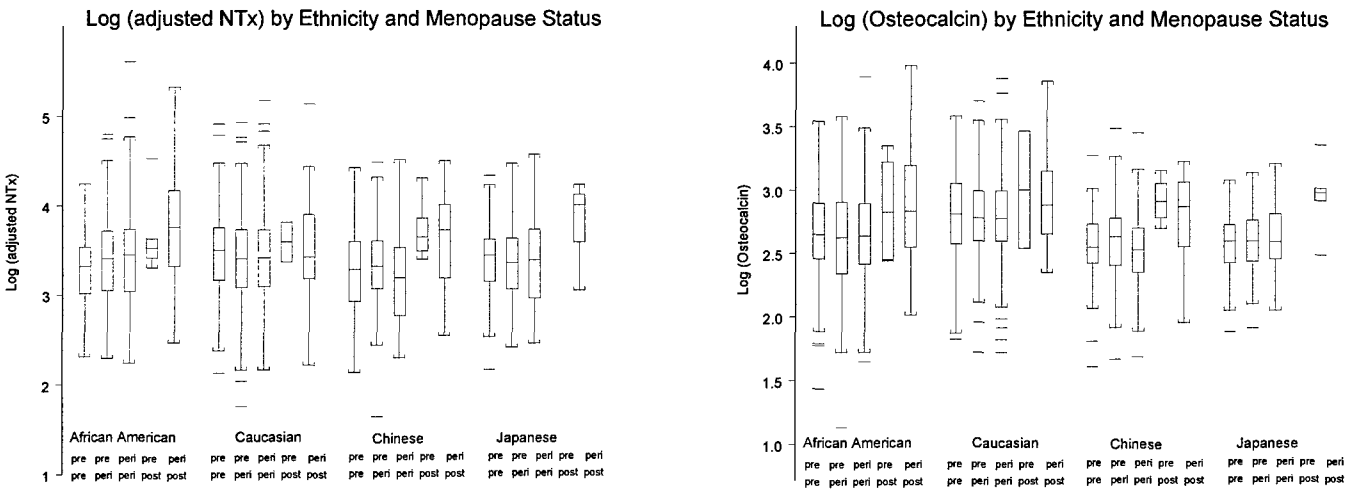
The analysis sample contains 194 women who are smokers at baseline (1331 are non-smokers). Using the median split method described in Aim 2 Part 2, no results are statistically significant for either smokers or non-smokers except for change in FSH preceding change in osteocalcin. For non-smokers, 55% of the

results are concordant (either both above or both below the median change for both FSH and osteocalcin, p=.0003), while for smokers only 46% of results are concordant (p=.31). Using a general linear model adjusting for visit 1 osteocalcin, baseline FSH, baseline age, and time since baseline, a smoking status by change in FSH interaction was not statistically significant (p=.21). Stratifying by baseline smoking status, we noted that among non-smokers, change in FSH from baseline to visit 1 was directly associated with change in osteocalcin from visit 1 to visit 2 (slope estimate=.0245, SE=.0044, p<.0001), while change in FSH was unassociated with osteocalcin change among smokers (slope estimate=.0013, SE=.0162, p=.93). So it may be that change in FSH is associated with later change in osteocalcin for non-smokers only, although the sample size for smokers is relatively small. Smoking does not alter relations between other hormone changes and bone marker changes.

AIM V: *To characterize bone turnovers in pre- and perimenopausal women belonging to various racial/ethnic groups and to determine whether observed differences can be accounted for by racial differences in specific lifestyle factors.*

Do premenopausal African American women have lower serum osteocalcin levels and urinary NTx excretion at baseline than Caucasian or Asian women?

Figure 9. NTx and Osteocalcin by Ethnicity and Menopause Status



This section considers whether baseline osteocalcin and NTx levels differ by ethnic group, before and after controlling for lifestyle factors: current smoking, calcium intake, and weight. Models also control for baseline age and status (pre or peri-menopausal). Because baseline bone markers tend to be right-skewed, models were fit using all the data on the original scale, "trimmed" data on the original scale (without some of the largest and smallest values), and logged data- this last is fairly normally distributed. Significance levels are quite similar all three ways. Results are reported using the full, untransformed data, except that p-values for osteocalcin contrasts on the log scale are shown in parentheses.

As hypothesized, African-American women have lower mean osteocalcin levels than Caucasian women at baseline (Figure 9 and Table 16), and Caucasians have higher mean levels than Asians. Unexpectedly, though, African-American women have higher mean levels than Asian women. Differences between Asian and non-Asian women increase when adjusted for smoking, calcium intake, and weight. Asians are lighter and smoke less than non-Asians. Because these factors are inversely related to osteocalcin, the effect is a reduction in adjusted compared to unadjusted means for osteocalcin in Asian women.

Table 16. Mean baseline serum osteocalcin level by ethnicity (ng/mL)

		N=1668	
		Unadjusted*	Adjusted
Ethnicity:	N	Mean (SE)	Mean (SE)
African-American	425	15.46 (.27)	16.12 (.28)
Caucasian	816	17.28 (.19)	17.34 (.19)
Chinese	208	13.97 (.38)	13.10 (.40)
Japanese	219	14.07 (.37)	13.35 (.39)

Contrasts:	Difference (SE)	p-value (log scale)	Difference (SE)	p-value (log scale)
A-A v. Caucasian	-1.82 (.33)	<.000 (<.0001)	-1.22 (.34)	.0004 (<.0001)
A-A v. Chinese	1.49 (.46)	.001 (.001)	3.03 (.51)	<.0001 (<.0001)
A-A v. Japanese	1.38 (.46)	.003 (.07)	2.77 (.50)	<.0001 (<.0001)
Caucasian v. Chinese	3.31 (.43)	<.0001 (<.0001)	4.25 (.45)	<.0001 (<.0001)
Caucasian v. Japanese	3.20 (.42)	<.0001 (<.0001)	3.99 (.44)	<.0001 (<.0001)

Covariates:	Slope estimates (SE)	p-value
Baseline smoking	-1.27 (.39)	.001
Baseline calcium (gm)	5.32 (3.70)	.15
Baseline weight (kg.)	-.048 (.007)	<.0001

* Adjusted only for baseline age.

Table 17. Baseline NTx by ethnicity (nM BCE per liter per mM creatinine per liter)

		N=1668	
		Unadjusted*	Adjusted
Ethnicity:	N	Mean (SE)	Mean (SE)
African-American	447	35.80 (.96)	36.43 (1.03)
Caucasian	817	35.39 (.71)	35.68 (.72)
Chinese	208	30.92 (1.40)	29.81 (1.48)
Japanese	220	32.91 (1.36)	31.58 (1.44)

Contrasts:	Difference (SE)	p-value (log scale)	Difference (SE)	p-value (log scale)
A-A v. Caucasian	.41 (1.19)	.73 (.65)	..75 (1.25)	.5 (.95)
A-A v. Chinese	4.89 (1.70)	.004 (.003)	6.62 (1.89)	.0005 (.0001)
A-A v. Japanese	2.90 (1.67)	.08 (.40)	4.85 (1.85)	.009 (.04)
Caucasian v. Chinese	4.47 (1.57)	.005 (.0004)	5.87 (1.66)	.0004 (<.0001)
Caucasian v. Japanese	2.48 (1.54)	.11 (.21)	4.10 (1.62)	.01 (.02)

Covariates:	Slope estimates (SE)	p-value
Baseline smoking	1.19 (1.43)	.41
Baseline calcium (gm)	-9.11 (9.09)	.32
Baseline weight (kg.)	-.087 (.027)	.002

* Adjusted only for baseline age.

Results for NTx are similar to those for osteocalcin (Figure 9 and Table 17), except that mean baseline adjusted NTx levels do not differ significantly for African-American and Caucasian women. Once again, Asian means are less than African-American means (although if one corrects for multiple comparisons, the Japanese result is not statistically significant). The lifestyle covariates, especially weight, not only do not explain unadjusted differences, but adjustment for them enlarges the unadjusted differences. Hence, neither weight, calcium intake, nor smoking, appear responsible for ethnic differences in baseline bone markers.

Change in bone markers from baseline to follow-up 02 by ethnic group, with status transition, age, weight, and smoking

This section considers whether a two-year change in bone markers differs by ethnicity, both before and after considering menopause transition category, age, weight, and smoking at baseline. To make model residuals approximate a normal distribution, a small number of "outliers" were "trimmed" from the data. For change in NTx, even trimmed data residuals are right-skewed, so models with an outcome of difference in log NTx were fit as well. Model results are reported for the trimmed untransformed data; for NTx, ethnic contrast significance levels are reported using both untransformed and log NTx.

Results (Tables 18 and 19) are reported in 3 columns: unadjusted (except for baseline bone marker), adjusted for transition category (and baseline bone marker), and adjusted for transition category, baseline bone marker, baseline age, weight, and smoking status, change in weight since baseline, and years since baseline.

Table 18. Change in osteocalcin from baseline to follow-up 02 (ng/mL), by ethnicity

		N=1502		
		Unadjusted	Adjusted for status	Adjusted for status, covariates
Ethnic group:	N	Mean (SE)	Mean (SE)	Mean (SE)
African-American	353	.47 (.24)	.29 (.24)	.80 (.25)
Caucasian	743	1.39 (.17)	1.54 (.17)	1.62 (.17)
Chinese	197	1.94 (.33)	1.83 (.32)	1.19 (.33)
Japanese	209	1.96 (.32)	2.01 (.31)	1.43 (.32)

Contrasts	Difference (SE)	p-value	Diff. (SE)	p-value	Diff. (SE)	p-value
A-A v. Caucasian	-.92 (.30)	.002	1.25 (.29)	<.0001	-.83 (.30)	.005
A-A v. Chinese	1.47 (.41)	.0003	1.54 (.40)	.0001	-.39 (.44)	.37
A-A v. Japanese	-1.49 (.40)	.0002	1.72 (.39)	<.0001	-.63 (.43)	.14
Caucasian v. Chinese	.55 (.37)	.14	.29 (.36)	.43	.44 (.38)	.25
Caucasian v. Japanese	.58 (.37)	.11	.47 (.36)	.19	.20 (.37)	.59

Transition category	N	Mean (SE)	Mean (SE)
PRE-PRE	311	-.06 (.25)	.17 (.25)
PRE-PERI	571	1.13 (.19)	1.12 (.18)
PERI-PERI	557	1.76 (.19)	1.73 (.18)
PRE-POST	9	5.59 (1.48)	5.17 (1.46)
PERI-POST	54	5.73 (.61)	5.17 (.61)

Covariates:	Slope est. (SE)	p-val	Slope est. (SE)	p-val	Slope est. (SE)	p-val
Baseline osteocalcin	-.11 (.023)	<.0001	-.14 (.022)	<.0001	-.15 (.022)	<.0001
baseline age					.22 (.045)	<.0001
years since baseline					2.37 (.86)	.006
baseline weight (kg)					-.03 (.0065)	<.0001
change in weight					-.10 (.024)	<.0001
baseline smoking					-.59 (.35)	.09

In the unadjusted model, African-Americans have a lower mean osteocalcin increase than do other ethnicities (Table 18). Adjusting for menopause status transition increases the differences, because more African-Americans are in the later transition groups, and these groups have higher mean osteocalcin changes. Adjusting for status, as though all groups have equal status, therefore, reduces the adjusted mean African-American osteocalcin increase. Adjusting for other covariates diminishes ethnic differences; African-Americans still have a smaller mean osteocalcin increase than Caucasians, but other differences are no longer statistically significant. African-Americans in the study are on average much heavier than Asians. Because weight is inversely associated with osteocalcin change, in a model with weight as a

covariate where different groups are "made equal" with respect to weight, Asians means are increased and the African-American mean is reduced.

Table 18. Change in NTx from baseline to visit 2 by ethnicity (nM BCE per liter per mM creatinine per liter)

		N=1547		
Ethnic group:	N	Unadjusted Mean (SE)	Adjusted for status Mean (SE)	Adjusted for status, covariates Mean (SE)
African-American	375	.19 (.78)	-.31 (.77)	1.07 (.81)
Caucasian	754	2.65 (.55)	2.92 (.54)	3.18 (.54)
Chinese	204	4.52 (1.06)	4.20 (1.04)	2.46 (1.08)
Japanese	214	3.67 (1.03)	3.98 (1.02)	2.26 (1.05)

Contrasts	Difference (SE)	p-value	Diff. (SE)	p-value	Diff. (SE)	p-value	log(NTx) p-value
A-A v. Caucasian	-2.46 (.96)	.01	-3.23 (.94)	.0006	-2.11 (.96)	.03	.003
A-A v. Chinese	-4.33 (1.32)	.001	-4.52 (1.30)	.0005	-1.38 (1.42)	.33	.07
A-A v. Japanese	-3.48 (1.30)	.007	-4.29 (1.28)	.0008	-1.18 (1.39)	.39	.22
Caucasian v. Chinese	-1.87 (1.20)	.12	-1.28 (1.18)	.28	.73 (1.23)	.55	.85
Caucasian v. Japanese	1.02 (1.17)	-.38	-1.06 (1.15)	.36	.93 (1.20)	.44	.35

Transition combination	N	Mean (SE)	Mean (SE)
Pre-pre	319	-1.15 (.83)	-.28 (.83)
Pre-peri	574	1.25 (.62)	1.28 (.61)
Peri-peri	582	4.13 (.62)	3.97 (.60)
Pre-post	13	16.57 (4.14)	14.40 (4.07)
Peri-post	59	12.41 (1.96)	10.28 (1.97)

Covariates:	Slope est. (SE)	p-value	Slope est. (SE)	p-value	Slope est. (SE)	p-value	log(NTx) p-value
Baseline osteocalcin	49 (.022)	-.<.0001	-.51 (.022)	<.0001	-.52 (.021)	<.0001	<.0001
baseline age					.81 (.147)	<.0001	<.0001
years since baseline					4.36 (2.79)	.12	.09
baseline weight (kg)					-.09 (.021)	<.0001	<.0001
change in weight					-.36 (.08)	<.0001	<.0001
baseline smoking					-.86 (1.12)	.44	.26

Results for change in NTx are similar. African-American means are lower than Caucasian means in all models, including that with covariates where the difference of log (NTx) is the outcome. Again, adding weight to the model increases the mean African-American NTx change while reducing the mean Asian change, leaving the Caucasian mean change relatively stable compared to the unadjusted mean change.

In conclusion, bone markers increase over the two-year period less for African-Americans than for Caucasians, even accounting for "lifestyle" factors (especially weight). Asian increases for both markers are larger than African-American increases, but after controlling for weight differences are not statistically significant.

7. Key Research Accomplishments and Reportable Outcomes

Baseline manuscripts for which writing groups are formed and currently working on analyses:

1. A comparison of ethnic differences in bone turnover (serum osteocalcin and urinary NTx excretion) among African American, Caucasian, Chinese and Japanese women. This paper will also include ethnic differences in bone mineral density and bone mineral apparent density.

2. Correlates of bone mineral density, bone turnover markers and menopausal status in SWAN
3. The relation of physical activity with bone mass and bone turnover in middle-aged women
4. The relation of phytoestrogen consumption with bone mineral density and bone turnover in a multi-ethnic cohort of mid-life women.
5. Correlations of bone mass with body size.

Writing groups to analyze longitudinal data will form in the coming year.

To date no manuscripts have been submitted for publication.

8. Conclusions

- Mean serum osteocalcin and urinary NTx levels increase with age and as women progress through the menopausal transition. This trend is consistent among all ethnic groups. A longer follow-up time is necessary to conclude whether menopause transition stage or elapsed time are more closely associated with increase in bone turnover marker levels.

- African American women have lower mean baseline osteocalcin levels than Caucasian women. Caucasian and African American women have higher mean levels than Asian (Japanese and Chinese) women.

- There is minimal predictive value of baseline bone markers for subsequent two-year bone loss. Association of bone turnover with bone loss becomes stronger with proximity to the end of the two-year observation period. Higher baseline NTx is somewhat associated with greater bone loss at the spine and total hip, but not the femoral neck. Baseline osteocalcin does not predict subsequent bone loss at any anatomical site. Concurrent bone marker measurements are more strongly associated with bone mineral density than prior measures.

- A change in E2 level early in the menopausal transition is not a good predictor of a future change in serum osteocalcin or urinary NTx, while change in FSH over the past year does help predict change in osteocalcin over the next year, and may, in conjunction with current NTx, help predict future change in NTx.

- Baseline bone turnover markers are not good predictors in premenopausal women of the transition to perimenopause. Bone markers do not predict the onset of the perimenopause better than ovarian hormone levels. Baseline level of FSH, but not estradiol, is a predictor of the subsequent transition to peri-menopause.

- Of the three lifestyle factors examined, smoking, calcium intake, and weight, only baseline weight and change in weight since baseline appear inversely related to change in bone turnover markers.

- Smoking does not alter the relations between hormone (FSH and estradiol) changes and bone turnover marker changes for women early in the menopausal transition.

- None of the lifestyle factors, weight, calcium intake, or smoking explain ethnic differences in levels of bone turnover markers.

- Bone markers increase over the two-year period less for African-Americans than for Caucasians, even accounting for "lifestyle" factors (especially weight). Asian increases for both markers are larger than African-American increases, but after controlling for weight differences are not statistically significant.

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Personnel on this project

The following personnel received salary support from this research effort:

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Sonja McKinlay, PhD	Principal Investigator
Sybil Crawford, PhD	Co-Principal Investigator, Senior Statistician
Joel Finkelstein, MD	Co-Investigator
Beth Willis	Senior Data Manager
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REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 13 Feb 02

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

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2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLIS M. RINEHART
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